

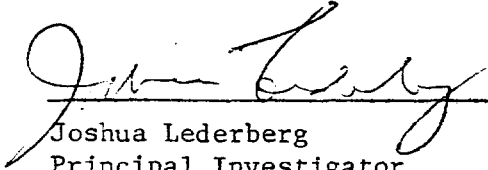
BIOCHEMICAL MARKERS OR ENZYME CHANGES THAT MAY PRESAGE THE PRESENCE OF CANCER

CONTRACT NUMBER NO1-CB-43902

SEMI-ANNUAL REPORT (February 1, 1975 to June, 30, 1975)

Submitted by:

THE BOARD OF TRUSTEES OF THE LELAND STANFORD, JR., UNIVERSITY



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Progress Report for the Period February 1, 1975 to June 30, 1975

A. Development of An Analytical Method for the Quantitation of Urinary Polyamine Levels.

We have been successful in developing a method for the isolation of polyamines from urine. This procedure uses an initial butanol extraction of alkaline urine followed by further purification using ion exchange chromatography. The gas chromatography separation of isolated polyamines remains as described in our semi-annual report (July 74 to January 76) and specifically labelled deuterated polyamines are used as internal standards.

The following description reflects the current state of the isolation procedure we use and no further development is anticipated to be required for this phase of the operation.

Isolation of Polyamines by Mass Fragmentography.

50 ml of urine are hydrolyzed overnight with 6N hydrochloric acid. A specific amount of deuterated polyamines (putrescine, cadavarine, spermidine spermine) is added to the urine prior to hydrolysis overnight. The reaction mixture is cooled to room temperature and filtered. This filtered solution is extracted with three volumes of 1:1 ethyl acetate/ether and the organic phase is discarded. The aqueous phase is taken to dryness and the residue is dissolved in a solution saturated with 3g sodium sulfate plus 1g sodium phosphate. The solution is now made pH 14 and extracted with 3 volumes of n-butanol. The aqueous layer is discarded. The butanol phase is filtered, acidified with 6N hydrochloric acid, and taken to dryness. The residue is reconstituted in water and adjusted to pH 4.5-5.0. The solution is filtered and the filtrate placed onto an ion exchange column (Dowex 50, H⁺ from 87% cross linked, 200-400 mesh). The eluant is discarded and the column is washed with 80 ml of buffer (0.1M in sodium hydrogen phosphate and 0.7M in sodium chloride and pH 8.4) and the eluant again is discarded. The column is eluted with 25ml of 1N hydrochloric acid followed by 20 ml of 6N hydrochloric acid. Only the latter washing is saved.

The 6N hydrochloric acid washings are dried in vacuo and derivatized by heating with trifluoroacetic anhydride in methylene chloride solution. Excess reagent is removed in a stream of dry nitrogen and the residue is dissolved in 25 μ l of ethyl acetate and 1 μ l is injected into the GC/MS system (column 10% OV-17, 6 ft. initial temperature 130°C, flow rate 30 ml/min.). After two minutes the column is programmed at 8°C/min and after 7 minutes mass spectral data acquisition is commenced.

At the present time the response of the GC/MS system using mass fragmentography for the quantitative detection of known amounts of deuterated and non-deuterated polyamines has been determined. Using this information we have constructed standard curves for the quantitation of indigenous polyamines in urine from cancer patients.

B. Screening of Urine for Metabolites which might Presage the Presence of Cancer.

During the period covered by this report we have completed GC/MS profiles on the following distribution of cancer patients and controls.

bladder cancer	1 (total of 9)
non-Hodgkin lymphomas	3 (" " 9)
cancer of the prostate	1 (" " 4)
leukemias	0 (" " 7)
Hodgkin's	5 (" " 5)
controls	5 (" " 5)

In addition we have expanded our analytical screen to include volatile fatty acids which would have been eliminated in the fractionation scheme in use. The method used was to inject acidified urine directly onto an appropriate gas chromatography column to detect the following fatty acids: acetic, propionic, n- and isobutyric acids and the valeric acids. In all the samples/controls studied we failed to detect any of these fatty acids and conclude that these compounds are not excreted in cancer urines in concentrations of significance for cancer diagnosis.

No significant differences were found in the compound distribution of those urines analyzed during this past reporting period and that included in the first report of this contract's progress. We have ascertained that the unidentified component present in a number of cancer urines is also found in all the control urines we have investigated. We did attempt to identify this compound, believing it to be either a pyrrole-2 or -3 carboxylic acid. However, the mass spectra of these two compounds were different from that of the unknown which remains unidentified.

We have observed that beta-aminoisobutyric acid (BAIB) is a frequent constituent of the majority of cancer urines examined to date. Recent improvements to our analytical methodology (accomplished in relation to other sponsored research) will make it possible for us to quantitate for BAIB using a modification of our previous analytical procedure (Clin. Chim. Acta, 49, 401 (1973)). Accurate quantitation of BAIB is essential in view of the separation of the concentration levels of genetic excretors of BAIB from patients afflicted with neoplastic disease.

Personnel	Title	Soc. Sec. No.	% Time
(a) Lederberg, Joshua	Professor of Genetics	[REDACTED]	5%
Duffield, Alan M.	Research Associate	[REDACTED]	40%
Everhart, Edwin T.	Research Assistant	[REDACTED]	100%
Anderson, Patricia	Research Assistant		75%(May 12-June 30)

(b) To date work under this contract is in fact-building stage and has not yet resulted in the publication of any scientific research papers.

(c) No invention reports resulted from work sponsored by the contract.